

THE IMPACT OF AGE AND EXERCISE ON STRESSED MICE

A Thesis

Presented to the

Faculty of the College of Graduate Studies and Research

Angelo State University

In Partial Fulfillment of the

Requirements for the Degree

MASTER OF SCIENCE

by

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May 2021

Major: Experimental Psychology

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ABSTRACT

Adolescence is a period that is often accompanied by increased stress levels, leading to negative effects on learning and memory. Stress during critical periods of brain development, including adolescence, can often lead to negative future memory performance. Adolescents are not the only ones affected by stress; negative results in learning and memory have also been seen within adult populations. The current study aimed to explore whether age and exercise had an impact on learning and memory in stressed mice. Adolescent mice expressed less anxiety-related behaviors and had higher locomotor activity compared to adults in the Open Field (OFT) behavioral test ($p < 0.05$). Adults who exercised expressed more risk taking behavior within the Elevated Zero Maze (EZM) ($p < 0.05$). However, adults showed better learning and memory results compared to adolescents in the Morris Water Maze (MWM) behavioral test ($p = 0.054$). Adolescents that exercised showed less risk-taking behaviors but had more defensive behaviors seen through the burrowing assay (ADL) measure ($p < 0.05$). The findings from this study showed agreement with previous literature. However, there were several instances where previous findings did not match with what was found in the current study. Future research should investigate the impacts that age and exercise have on mice throughout the lifespan; this will aim to fill in gaps present within the literature.

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INTRODUCTION

Adolescence is a period that is often accompanied by increased stress levels. Increased stress leads to negative results in learning and memory, including long term cognitive deficits (Vogel & Schwabe, 2016). During critical periods of brain development, such as adolescence, learning and memory can be affected by stress, causing future memory performance to be negatively impacted (Schwabe et al., 2012). Researchers have found several benefits of exercise within adolescents and other age groups. These include mood enhancement (Streeter et al., 2010), increased brain health (Cotman et al., 2017), positive development (Mridula et al., 2017), increased attention span, increased working memory (Guiney & Machado, 2013), and more positive recoveries from substance abuse (More et al, 2018 and Sampedro-Piquero et al., 2020).

Age Differences

A study done by Streeter et al. (2010) suggests that there is a positive relationship between exercise and moods in adult individuals (ages 18-45). They suggest that the beneficial effects of exercise on mood and anxiety are due to increased levels of GABA, measured as a function of exercise. Streeter et al. (2010) also showed that exercise can improve mood and decrease stress within young adults. Similarly, research by Cotman et al. (2017) suggests that exercise targets many aspects of brain functioning and has many positive effects on the elderly's overall brain health from increasing synaptic plasticity to the reduction of inflammation.

Cotman et al. (2017) focused on the benefits of exercise to learning and memory in the elderly community. They found that aerobic exercise or endurance type exercises, increased synaptic plasticity by positively affecting synaptic strength. This is important because the belief is that positive improvements in learning and memory can occur through strengthening neuronal plasticity and other functions including metabolism and vascular functioning. Cotman et al.

(2017) also found that exercise reduced inflammation, which can lead to a reduction in various risk factors and ensure successful brain functioning (Cotman, 2017). This research supports the notion that starting exercise earlier on in life may help to reduce the risk of diabetes, hypertension, and cardiovascular disease, which can contribute to dysfunctions in the brain.

While Cotman (2017) and his colleagues focused on the effects of exercise within older populations, Mridula et al. (2017) focused on studying memory in a younger population. Mridula et al. (2017) studied the differences in memory improvements in earlier ages of adulthood (19–40 years) and compared them to middle aged adults (40–65 years). Mridula et al. (2017) focused on working memory in young and middle-aged adults by assessing different methods of memory training and studying their effects. The researchers evaluated the findings found within the different memory training methods and related them to the individuals' cognitive communicative abilities. In this study, Mridula et al. (2017) found that the young adults, ages 19 to 40 years old, had higher improvement rates in their training compared to the middle-aged adults, ages 40 to 65 years old (Mridula et al., 2017). Therefore, it was shown that age had a significant effect on memory improvement. This research stresses the importance of starting memory training at an early age to better enhance or re-establish an individual's cognitive abilities throughout their life. While many researchers have focused on memory in different age populations, none have specifically focused on stressed individuals in each different age population.

Positive Effects of Exercise

Relating memory and exercise, a review written by Guiney & Machado (2013) suggest that there are many functional benefits to exercise. In regards to the way individuals develop, Guiney and Machado (2013) discuss a relationship between individuals pursuing healthy lifestyles earlier in life and long term health benefits. These included improved task switching, Selective attention, inhibition of proponent responses, and working memory capacity to those

who pursued healthy lifestyles earlier in life (Guiney & Machado, 2013). Guiney and Machado's review suggests that working memory can benefit from cross-sectional fitness, and regular engagement in exercise can provide a means for individuals to strengthen their memory and overall health (Guiney & Machado, 2013).

Exercise also has positive effects within young adults struggling with substance abuse disorders. More et al. (2018), specifically evaluated how the benefits of exercise can help with exercise motivation and substance abuse recovery. The researchers found that not only did youth benefit by showing positive health outcomes, but also reported positive changes in routines, perceptions, sleep quality, relationships, cathartic effects, and feelings of self-accomplishment. The researchers suggest that the positive results listed may have been an essential aspect in regards to the success of substance use disorder treatment.

Similarly, Sampedro-Piquero et al. (2020) found that introducing physical activity during adolescence and early adulthood can mitigate certain neurobehavioral alterations associated with binge-drinking, such as anxiolytic-related behaviors, preference for alcohol, and self-cleaning behaviors. Findings from Sampedro-Piquero et al. (2020) correspond with previous literature which stresses the importance of introducing exercise during adolescence.

Exercise as a Stressor

Although exercise can lead to decreased stress effects, in rodent models it can also become a stressor. Svensson et al. (2016) found that exercise induced stress in mice. Exercise could become a stressor in mice due to the inability to become fully habituated to the exercise apparatus prior to starting the given test protocol. Stress impacts learning and memory; therefore, it is critical to evaluate the effects of behavior when stress is encountered during critical development periods, such as adolescence.

Stress in Adolescence

As an adolescent grows and develops, stress is often introduced. Some of these stressors include school demands and frustrations, changes within their environments, body development, difficulties with peers and parents, and heightened expectations (Aacap, 2019). It is important to aid adolescents with these stressors by providing them with good coping mechanisms such as physical activity.

Purpose/Hypothesis

This project serves as a baseline to understand the impact of age and exercise on the body when chronic mild unpredictable stress (CMUS) is encountered within mice. Exercise is linked to positive health results while stress is linked to negative health results (Hei et al., 2019 & Vogel and Schwabe, 2016). By addressing the lack of research assessing the effects of exercise on adolescent and adult mice that begin receiving CMUS, this project aids in understanding how exercise combats stress-induced effects and whether there is an optimal time to introduce exercise when stress is encountered.

To evaluate the significance that age and exercise have on the body when stressors are introduced, mice were assigned into 4 different groups: exercise at adolescence (4 weeks), exercise at adulthood (14 weeks), no exercise at adolescence (4 weeks), and no exercise at adulthood (14 weeks). All groups experienced CMUS, with exercise and age being the variables changed among the mice. This allowed for the assessment of how learning and memory would be impacted by stress and how age and exercise may change the effects of stress. All groups went through behavioral tests that assessed daily living behaviors (burrowing and nesting), learning and memory, and anxiety measures. The hypothesis was that adolescent mice that underwent stress but were given exercise would have significantly better results than those not exposed to exercise and only had the stressors. These adolescents were hypothesized to have less anxious tendencies and better outcomes in measures of learning and memory.

METHODS

Subjects

48 C57BL/6J mice were purchased from The Jackson Laboratory (Jax, Bar Harbor, ME). There were 24 adolescent-aged mice (12 male and 12 female) at 4 weeks old. The remaining 24 mice were adults (12 male and 12 female) at 14 weeks old. The males and females were both housed 3 to a cage in an Animal Care System Optirat© rodent caging system. The mice were handled routinely during the week to ensure they were habituated to human touch. This was done to reduce the chance that being handled would become a stressor. Health and welfare checks were done routinely to ensure animals remained healthy. Adolescent mice had ear punches for identification purposes, while the adult mice were identified using tail markings. The tail markings were made using a non-toxic marker. Tails were marked frequently to ensure visibility and to prevent the mice removing the markings through grooming. Two cohorts were utilized to allow for fewer animals to be run during behavior testing, allowing for shorter testing days. Each cohort began with 24 mice: 12 adolescent mice, and 12 adult mice. One mouse was excluded from each cohort due to established exclusion criteria (see section Exercise protocol). The methods were identical amongst both cohorts. After the animals had acclimated to the laboratory space, the mice were randomly assigned to either the no-exercise or the exercise condition.

Exercise protocol

Habituation (One week)

This project required all mice to first partake in a week of habituation to the treadmill to attempt to reduce the risk of exercise becoming a stressor. The habituation protocol used was the same as that presented by Marques-Aleixo et al. (2015). The protocol began with the treadmill from Harvard Apparatus off (speed 0 cm/sec). Mice were individually placed on the separate lanes of the mouse treadmill, one cage at a time. The mice were then allowed to

explore the treadmill for one minute prior to the treadmill being turned on. Following the minute of the treadmill being stationary, the treadmill was then turned on slowly, increasing the speed until it began moving. The treadmill was only turned on for the groups of mice who were randomly selected to be in the exercise group. The non-exercise group remained on the stationary treadmill for the duration of time. The speed of the treadmill for the exercise group was set to 25cm/sec for 10 minutes. The exercise mice were able to explore the treadmill for an additional one minute after the 10 minute trial was complete. The mice were then returned to their home cages. The treadmill was cleaned with 70% ethanol after each cage completed its trial. The treadmill was allowed to dry prior to the start of the next cage's trial. The habituation protocol was repeated every day, Monday to Friday, at 5pm (3 hours before the dark cycle) for the duration of one week.

Treadmill Exercise (Three weeks)

The mice in the exercise condition then continued onto the treadmill exercise routine following the habituation period. The mice in the no-exercise group continued to be placed on the stationary treadmill during this time. Animals were placed on the treadmill, with the speed increasing from 33cm/s to 35cm/s over a three week period, running for 30 minutes each day. Week 1 consisted of treadmill speeds of 33cm/s for 30 minutes for 5 consecutive days (Monday to Friday). Week 2 consisted of treadmill speeds of 35cm/s for 30 minutes for 5 consecutive days. Week 3 also consisted of 35cm/s for 30 minutes for 5 consecutive days. The shock bars were turned on for the three week exercise paradigm in order to facilitate running. The shocks stayed at a consistent 0.8 milliamps (mA) for the first two weeks and were increased during the third week to 1.3mA to ensure the animals were running at a consistent pace. Mice that were unable to complete 20% (6 minutes) of the total 30 minute protocol for five consecutive days were deemed

“bad runners” and were not included in any behavioral data analysis. Mice were weighed on the first day of the week (Monday) and the last day of the week (Friday) during the three week exercise period. The same cleaning procedures performed during habituation were performed in between each trial throughout the exercise protocol. Trials started at 5pm each day, similar to the habituation period. The order that the mice went onto the treadmill was randomized each day. All mice underwent chronic stress alongside running exercise or stationary treadmill exposure for the three week exercise period.

Chronic Mild Unpredictable Stress (CMUS) Protocol

All animals experienced two stressors each day for the three week period (Table 1). The CMUS paradigm had been adapted and modeled from other CMUS protocols (Lippi, 2021, Han et al., 2016, DeVallance et al., 2017, Monteiro, et al., 2015). The order of stressors overall and per day were randomly assigned across the three week period. The three week period included Monday through Friday, totaling 15 days of stressors. The mice experienced the following stressors: Predator urine, no bedding, tilted cage, altered light cycle, bath, damp bedding, forced swim, bright light/open field exposure and overnight water deprivation (Table 1). The order of stressors the animals were involved in were randomized each week, ensuring the animals did not have the same stressor two days in a row.

Stressor	Procedure
Forced Swim	Animals were placed in cold water to swim (8-10C) for 5 minutes.
Overnight Water Deprivation	Animal's water bottles were removed right before lights out (8PM) and were replaced at lights on, the following morning (8AM).
Bright Light/Open Field Exposure	Animals were placed on an elevated platform (Barnes Maze apparatus) inside an open field container with 2 overhead lights shining down on them for a period of 10 minutes.
Altered Light Cycle	During the dark phase, animals were transported to the testing room and were kept in 'lights on' for the evening, with the room remaining lit. At the conclusion of the dark phase (8AM), animals were then placed back on the caging racks in the vivarium.
Damp Bedding	The animal's bedding was soaked with water and left in the cage for a period of 2 hours.
No Bedding	The animal's bedding was taken out for a period of 2 hours.
Tilted Cage	The animal's home cages were tilted for a one hour period.
Predator Urine	The animals were placed in a cage where 1mL of bobcat urine was placed on a cotton ball in a falcon tube in the cage with the mice for a duration of one hour.
Bath	The mice were placed in a cage with no bedding, with a small amount of water covering the cage floor for a period of two hours.

Table 1: List of Stressors according to the Chronic Mild Unpredictable Stress protocol.

Behavioral Measures

All mice underwent a series of behavioral tests to investigate the following: spatial memory, non-cognitive daily living behaviors (burrowing and nesting), locomotion and anxiety-related behavior. The sample sizes for each specific behavioral measure are presented in Table

2.

	OFT	EZM	MWM	ADL
Exercised Adults	n=4	n=4	n=4	n=2
Exercised Adolescents	n=6	n=6	n=6	n=3
Non-exercised Adults	n=6	n=6	n=6	n=3
Non-exercised Adolescents	n=6	n=6	n=6	n=3
	n=22 Females		n=24 Males	

Table 2. Experimental sample sizes for each behavioral analysis; two females were removed based on exclusion criteria.

The Open Field Test (OFT)

The Open Field Test (OFT) evaluates novel environment exploration, general locomotor activity, and provides a screen for anxiety-related behavior within mice and rats (Seibenhener & Wooten, 2015). The animals were individually placed in a white plastic box, measuring 45cm x 45cm x 40cm for a five-minute trial where the following variables were measured by an overhead camera with SMART animal behavior software (Panlab, Harvard Apparatus): time spent in the center vs. the surround, latency to first enter into the center of the box, and total distance travelled.

Elevated Zero Maze (EZM)

The Elevated Zero Maze (EZM) is a behavioral test that assesses anxiety-like behavior in mice (Kulkarni, et al., 2007). The apparatus is approximately 40 inches above the ground. The maze consists of a “0” shape containing two closed arms (containing high walls) on opposite sides and two open arms (no walls). The EZM apparatus allows the mouse to travel between open, unprotected maze arms and the enclosed protected arms. Mice were determined to be in either the closed or open arm when all four paws were inside that given area. Naturally, mice tend to avoid unprotected areas and spend more time in protected enclosed areas, allowing for researchers to evaluate anxiety-like behaviors. Each trial lasted five minutes and the following variables were measured: time spent in the open v. closed arms, number of transitions, and head

dips assessing risk-taking behavior.

Morris Water Maze (MWM)

The Morris Water Maze (MWM) is a behavioral test that assesses spatial memory in rodents (Morris, 1984). Mice learn to find a clear platform hidden just below opaque water using visual cues surrounding a tub. The test consists of 3 trials per day, for a 5 day period. Animals were given 60 seconds in each trial to find a hidden platform; if animals were unable to do so by the end of the trial they were guided to the platform and allowed to stay on it for 10 seconds. The mice use visual cues, hung around the outside of the tub, to find and learn the location of the platform. The variables measured in each trial included: latency to reach the platform (seconds), target crossings on days 2 and 4, entries into the target quadrant, and time spent in the target quadrant. These measures were recorded by use of an overhead camera connected to SMART animal behavior tracking software (Panlab, Harvard Apparatus). On days 2 and 4, the third trial was a probe trial where the platform was lowered to where the animals could not access it. The mice were given 60 seconds before the platform was raised again. Probe trials were used to measure the time that the animal spends looking for the platform in the target quadrant.

Activities of Daily Living (ADL)

Activities of Daily Living (ADLs), such as burrowing and nesting, assess animals' natural instincts to seek shelter for heat and safety (Deacon, 2013). When measuring the animals burrowing tendencies, a PVC tube with one end closed was filled with 250 grams of pea gravel and placed in an individual cage with the mouse. The PVC tube was weighed after 2 hours and the following morning to document how many grams of pea gravel had been removed. Nesting behavior was assessed by scattering 3 grams of shredded white paper across the cage. The nests that were constructed by these mice after 24 hours were then scored by four raters who were blind to experimental conditions, rating the nests on a 1 to 5 point scale. The scale ranges

from one, paper appearing to be untouched to five, where the mouse used all of the paper provided to build a nest. Analysis was done on the average nesting score for each cage. Intraclass correlation for reliability was also assessed to ensure each of the rater's scores was reliable.

Statistical Analysis

Data collected from the Morris Water Maze was analyzed using a mixed ANOVA with age and exercise (between-subjects variable) and days (within-subject variable) as the factors. Data collected from the OFT, EZM, and ADL (burrowing and nesting) were analyzed using factorial ANOVAs (2*2 design) (factors: age and exercise). Sex was run as a factor in analyses and was found to be non-significant; therefore, data were collapsed across sex.

RESULTS

Animal Weights

A within-subject effect of time (weeks) was shown, $F(1,42) = 48.695$, $p < 0.001$.

Animals' weights increased as the weeks went on throughout the experiment. There was also a significant interaction between weeks and age, $F(1,42) = 7.150$, $p < 0.05$. The between-subjects effect of age showed that adult mice weighed significantly more in comparison to the adolescent mice $F(1,42) = 65.059$, $p < 0.001$, partial $\eta^2 = 0.608$. There was no effect of exercise on recorded body weight.

Open Field Testing (OFT)

There was a significant effect of age on the total distance traveled within the OFT, $F(1,42) = 8.94$, $p < 0.05$, partial $\eta^2 = .176$ (Figure 1). Adolescents traveled significantly more than adults (Adult: $M = 2073.18$, $SD = 550.68$; Adolescent: $M = 2530.37$, $SD = 481.12$).

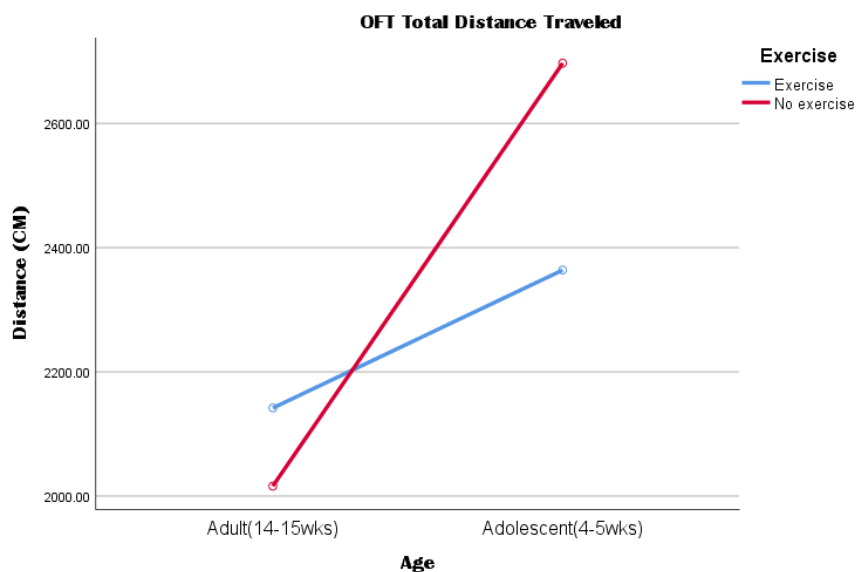


Figure 1. Total Distance traveled within the Open Field Test

There was a trending effect of exercise on the percent of time within the surround of the OFT box, $F(1,42) = 2.857$, $p = 0.098$, partial $\eta^2 = 0.064$. The exercised group spent less time in the surround compared to the non-exercised group (Exercise: $M = 96.57$, $SD = 2.15$; No-exercise: $M = 97.45$, $SD = 1.22$).

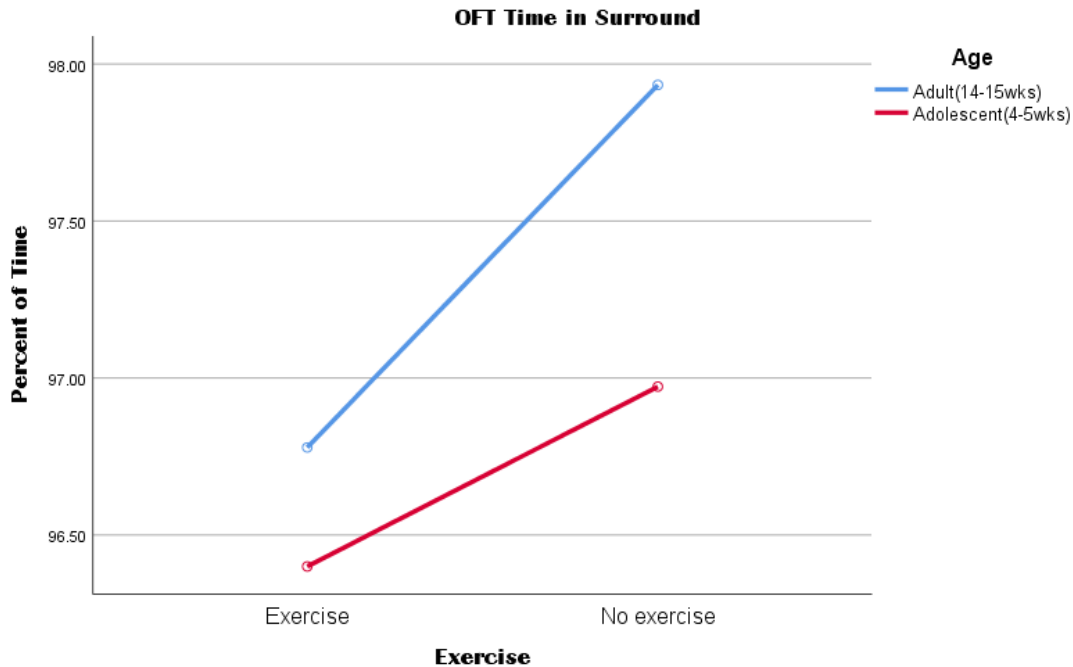


Figure 2: Time spent in the Surround of the Open Field Test

There was a trending effect of exercise seen in the percent of time spent in the center of the OFT box, $F(1,42) = 2.857$, $p = 0.098$, partial $\eta^2 = 0.064$. The exercised group spent more time in the center in comparison to the non-exercised group (Exercise: $M = 3.42$, $SD = 2.15$; No-exercise: $M = 2.55$, $SD = 1.22$).

Elevated Zero Maze (EZM)

There was a significant interaction between age and exercise for the number of head dips

made in the EZM, $F(1,42) = 4.662$, $p < 0.05$, partial $\eta^2 = 0.10$. Adults who exercised had significantly more head dips than adults who did not exercise ($p = 0.012$). This meant that the exercised adults had higher risk taking behaviors. Although not significant, adolescents who exercised had less head dips than those who were not exercised (Exercised Adolescence: $M = 21$, $SD = 6.35$; Non-exercised Adolescence: $M = 22.17$, $SD = 7$) (Figure 3).

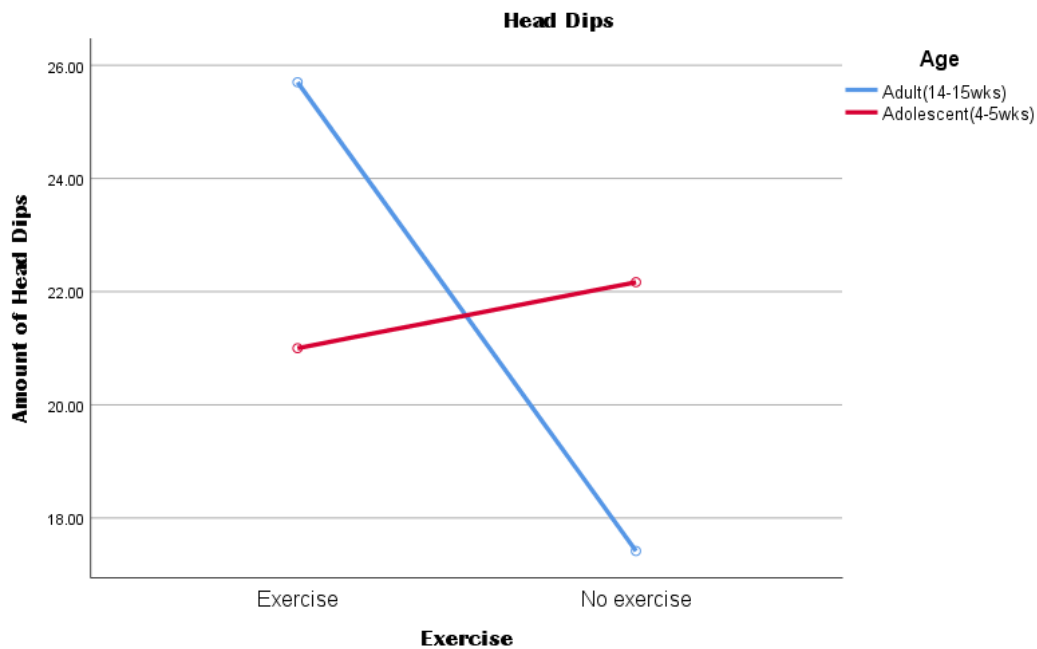


Figure 3: Head dips recorded by exercise vs. non exercise groups.

There was a trending Age*Exercise interaction for the number of transitions, $F(1,42) = 3.762$, $p = 0.059$, partial $\eta^2 = 0.082$. Adults who exercised had more transitions in the EZM compared to adults who did not exercise (Adults Exercise: $M = 5.9$, $SD = 3.41$; Adults No-exercise: $M = 5.08$, $SD = 2.68$). Simple effects analysis was used to investigate the interaction further and revealed a significant difference between the adolescents who exercised and those who did not exercise ($p < 0.05$). Adolescents who were in the non-exercised group had more

transitions than the exercised group. Therefore, more active behavior was noted within adolescents who did not exercise.

Morris Water Maze (MWM)

Latency to Find Platform:

While mice were able to learn the task over the five training days, as seen by a significant effect of day, $F(4, 168) = 10.317, p < 0.001$ there were no between-subject effects seen with age or exercise. By the fifth day of the MWM paradigm, the mice began to find the platform more quickly, leading to shorter latencies to find the platform (Figure 4A-C).

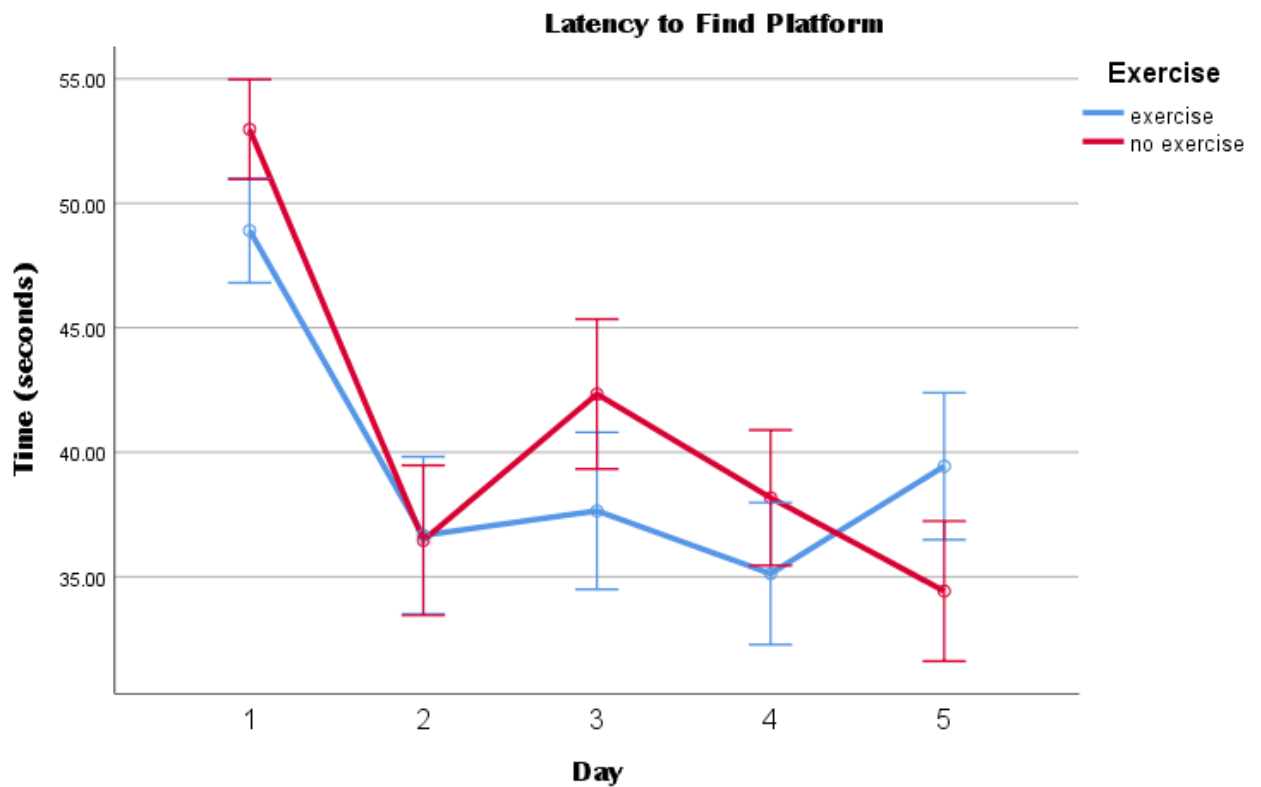


Figure 4A. Latency to Find the Platform over the five day MWM paradigm (Exercise).

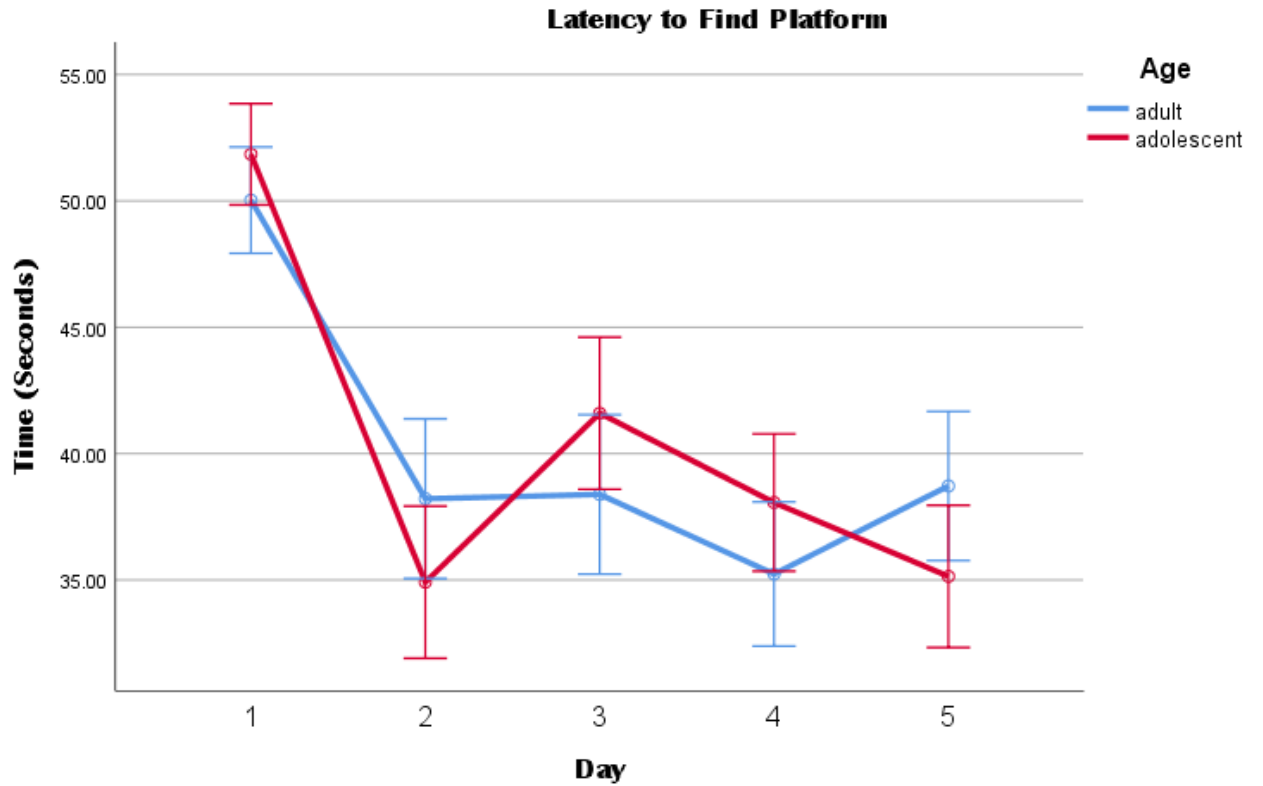


Figure 4B. Latency to Find the Platform over the five day MWM paradigm (Age).

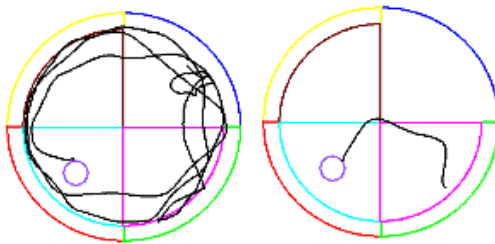


Figure 4C. Swimming paths to find the platform within the MWM. Representative image showing the progression between the first and last day.

Target Crossings:

There were no significant effects of age or exercise seen for the number of target crosses made on probe days 2 and 4.

Entries in Target Quadrant:

There was a significant effect of day for entries into the target quadrant, $F(4, 168) = 13.324, p < 0.001$. Throughout the five day paradigm, mice made more entries into the target quadrant as they learned where the platform was. There was also a trending interaction between day*age*exercise, $F(4, 168) = 2.098, p = 0.083$, partial $\eta^2 = 0.048$. The fourth day had a significant difference between exercised adults and exercised adolescence ($p < 0.05$), where the adults group had significantly more entries in comparison to the adolescent group.

Time in Target Quadrant (%):

There was no significance found for age and exercise on the amount of time (%) the mice spent in the target quadrant within the five day paradigm.

Activities of Daily Living (ADL)

Nesting

There were no significant effects of age or exercise on nests constructed by mice.

Burrowing

There was a significant effect of exercise seen for the 2-hour burrowing measurement, $F(1, 19) = 6.610, p < 0.05$, partial $\eta^2 = 0.258$ (Figure 5). Mice that exercised burrowed significantly more pea gravel than the no-exercise mice ($p < 0.05$). There was no effect of age for the 2 hour burrow measurement. There were no significant effects of age or exercise on the overnight burrowing measurement.

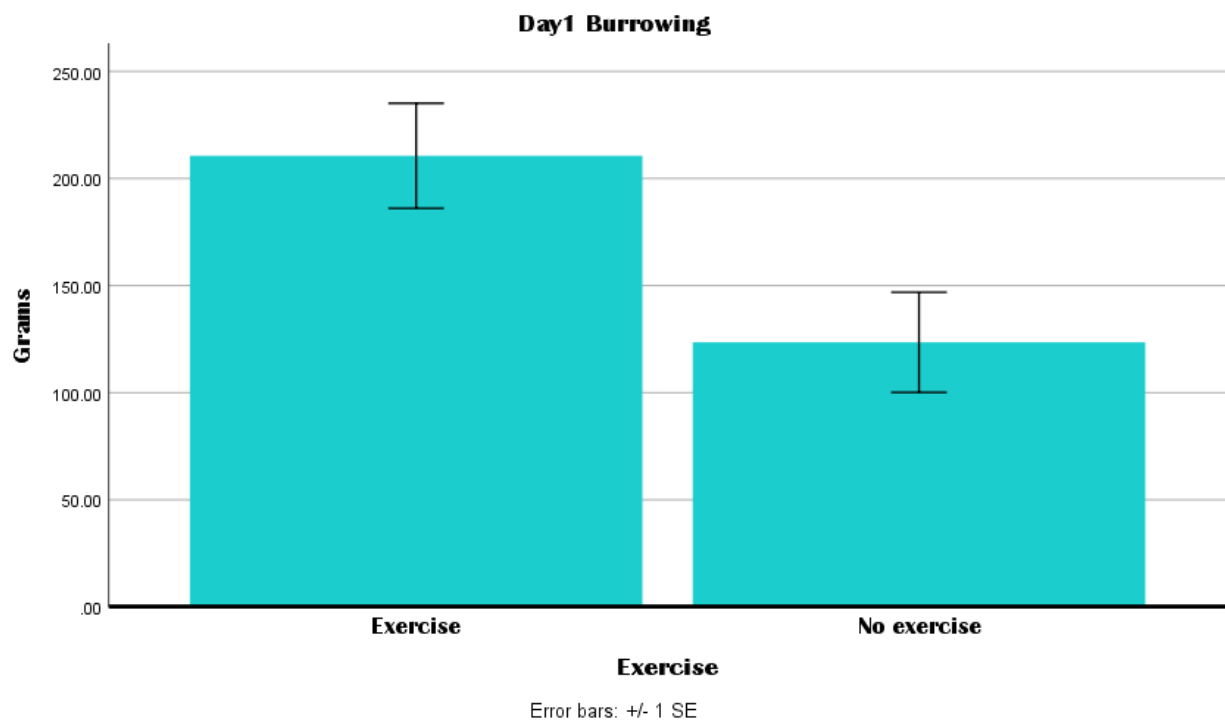


Figure 5. Burrowing patterns within exercised and unexercised groups.

DISCUSSION

The purpose of this study was to assess how age and exercise affect stressed mice; in particular, if they could improve any negative effects of stress. The hypothesis was that the exercise group would perform better within the behavioral tests, indicating lower anxiety tendencies and better learning and memory improvements, in comparison to those who were not exercising. The current study showed several different effects of age and exercise within the stressed mice. A few of the study's findings did not match previous findings that have assessed age and exercise as variables. However, these studies were not comparing these two variables on their effects specifically on stressed animals.

Behavioral Analysis

Animal Weights

There was a significant difference between adult weights and adolescent weights throughout the three week exercise/stress paradigm. This is to be expected as adults naturally weigh more than adolescents (Dutta & Sengupta, 2016). All weights increased throughout the paradigm. This is also to be expected because as mice age, an increase in weight is expected.

Open Field Testing

The results from the OFT show that adolescent mice travelled a significantly greater distance compared to adults. This indicates that adolescent mice are more prone to novel environment exploration, having more general locomotor activity based on distance travelled and exhibited less anxious tendencies (Green, Esser & Perrot, 2018). Green, Esser and Perrot (2018) found that adolescent mice did not avoid the center of the OFT test and signified that the adolescent mice showed less anxiety behavior in comparison to adult mice. Similarly, Neff and McGehee (2010) found that adolescents typically exhibited less anxious tendencies when compared to adults, specifically when evaluating individuals' self-compassion within humans.

These findings agree with the current study's finding that adolescents expressed more general locomotor activity and less anxious tendencies.

Mice that exercised spent more time on average in the center compared to the non-exercise group. Streeter et al. (2010) studied the impacts of increasing levels of GABA by exercise and how they affect mood and anxious tendencies. They found that exercise provided beneficial effects on mood through increasing the neurotransmitter GABA and relieving anxiety-related tendencies. This study relates to these findings, indicating that those who exercise may have less anxious tendencies. Perhaps this difference may be implicating the role of neurotransmitters like GABA in relieving anxiety within the mice.

Elevated Zero Maze

The results from the EZM indicated that adult mice who exercised exhibited significantly more head dips than adults that did not exercise. Head dips within the EZM indicate risk taking behavior. The adult mice who exercised exhibited more head dips; this shows a willingness to engage in risk taking behaviors, therefore, showing less anxiety-related tendencies. Streeter et al. (2010) and More et al. (2018) found that young adults who exercise are more willing to have motivation to exhibit new behaviors. This study's findings regarding exhibiting behaviors such as risk-taking behaviors are in agreement with the current study's findings of exercised adults through the EZM behavioral measure. On the contrary, the current study found that adolescents who exercised had less head dips than the adolescent mice who did not exercise, indicating that the adolescent exercise group did not engage in as much risk taking behavior. Adolescents are normally more willing to engage in risk taking behaviors in comparison to adults (Laviola, Macrì, Morley-Fletcher & Adriani, 2003). The current findings do not line up with this past research.

Adults that exercised had more transitions than adults who did not exercise. Adolescents who exercised had less transitions on average than adolescents who did not exercise. The findings

regarding adults transitions aligns with previous research that exercise leads to increased general movement (Guiney & Machado, 2013). However, the exercised adolescents showed less transitions, therefore exhibiting less general movement within the maze which does not align with previous research.

Morris Water Maze

The results from the Morris Water Maze did not line up with previous literature indicating that exercise has a significant positive effect on memory improvements (Cotman, Berchtold, and Christie, 2017). On average, although not significant, the exercised mice had less target crossing than the non-exercised groups, suggesting that the mice who underwent exercise exhibited worse memory improvements. This is opposite from what previous literature has shown. On average, results indicated that adults had more entries into the target quadrant compared to adolescents, although not significant. This is opposite of what Sampedro-Piquero (2020) and his colleagues found. These researchers found that introducing physical activity during adolescence can mitigate negative effects on behaviors. These behaviors include, positive alterations in anxiolytic-related behaviors and self-cleaning behaviors. Mridula et al. (2017), also found that younger populations (ages 19-40) show significantly better memory improvements in comparison to mature adult populations (ages 40-65). The current study's findings do not relate with previous literature in this regard.

Activities of Daily Living (Burrowing)

The results from the ADL assessments, specifically burrowing tendencies, showed a significant effect due to exercise. The mice given exercise burrowed significantly more than the no-exercise group. Burrowing is a natural defensive behavior shown in mice to protect themselves from possible predators. Burrowing behavior can be related to routine care within humans. The current study's findings relate closely with past research on exercise and the effects

of routine care. More et al. (2018), found that exercise can provide positive results within routine care within young adults struggling with substance abuse. He found that exercise helped with motivation within the young adults' recovery, leading to positive changes in routine care.

Limitations

The limitations within this study predominantly affected the MWM results. Specifically, within the first cohort. Due to unpredicted harsh weather conditions, the MWM data collection was cut short, resulting in only five days of data collection compared to the typical eight day paradigm. These weather conditions also prevented the first cohort from being run through ADL measures. The second cohort was also included in these harsh weather conditions which led to several power outages. These conditions could have potentially added more stress prior to habituating the second cohort to the treadmill. Additionally, the second cohort was given an extra week prior to habituating to the treadmill.

A limitation for the exercise group included the treadmill hardware positioning being adjusted. In order to prevent mice from laying on the shock bars, the treadmill hardware was adjusted to better encourage running. Another possible limitation for the exercise group was the attempt to refrain from the use of shocks. Shocks were initially not used during the habituation week; however, the shocks were deemed necessary during the first week of exercise and were used for the remainder of the paradigm. If shocks had been used from the beginning (habituation), this may have led mice to better associate the bars as an aversive stimulus, thus leading them to run more efficiently. Along with the treadmill shocks, the treadmill was also set to a slight incline, naturally pulling the mice towards the shocks through gravity. The treadmill incline could have affected the animal's ability and motivation to run. If the treadmill was set to a decline the animals would be naturally drawn toward the front of the treadmill away from the shocks assisting in the mice continually running forward.

Exercise may have become a stressor due to an inadequate amount of habituation time. Svensson and colleagues (2016) found that exercise introduced stress within mice. These researchers did not allow for a full week of habituation. However, even with the current study allowing for a full week of habituation it may have not been a sufficient amount of time, with too large of a change in pace in a short period of time (25cm/s for 10 minutes to 33cm/s for 30 minutes within a week). Most studies had longer durations of time allowing for an increased speed at a slower pace (Svensson et al., 2016, Kim, Choi, & Chung, 2016, Marques-Aleixo et al., 2015, Li et al., 2013, Liu et al., 2011, Luciano da Silva et al., 2009, Castro & Kuang, 2017, Dougherty, Springer, Gershengorn, 2016, Poole et al., 2020).

Since adolescent mice were found to weigh significantly less than the adult mice, the current study suggests that the adult mice were much larger than the adolescents. The difference in size within the mice may have affected their stride length on the treadmill within the running group. Previous research has looked at stride frequency and found that running velocity development was affected by stride frequency within adolescents (Chatzilazaridis, Panoutsakopoulos & Papaiakevou, 2012). The development of stride frequency within adolescence may have needed more time to develop within their running ability in comparison to the adults who exercised. Another study suggested that older participants developed more positive responses to exercise in comparison to younger participants (Reich & Queatherm, 2020). This may relate to their stride development being more developed within adults in comparison to adolescents.

Another limitation of the current study was a lack of a true control group. All of the groups went through stressors, therefore some of the behavioral measures did not show significance. There may have been more significance shown if there were a non-exercised non-stressed group compared to the stressed groups. Vogel and Schwabe (2016) show that increased

stress can lead to negative results in learning and memory within children in a classroom setting compared to children that do not experience increased stress. Having a non-stressed group and comparing results with the stressed group would allow for the possibility of finding greater significance.

The current study collapsed the data from both female and male mice. This was done because there was no significant difference seen when sex was added as a variable. Raffington et al. (2020) found that adolescent females had higher cortisol secretions in response to stress and higher subjective stress responses. This research further explains that females', specifically adolescent females', memory is greatly modulated by stress experience in comparison to adolescent males (Raffington et al., 2020). The current study did not find any significance of sex. However, this may have been due to smaller sample sizes. Future studies could include increased sample sizes within each sex to allow for more statistical power.

Future Directions

Future research could focus on the impacts of a decline on the treadmill. The current study did not include a decline and because of the positioning of the treadmill there is a slight incline naturally pulling the animals back towards the shocks. By adding a decline the mice would be assisted by gravity towards the front of the treadmill away from the shocks.

Further research is needed to assess the optimal exercise paradigm that would lead to significance. The current study's exercise paradigm may have not been as drastic of a paradigm necessary to yield significant results. While the current study's exercise paradigm is modelled after a combination of other paradigms (Svensson et al., 2016, Kim, Choi, & Chung, 2016, Marques-Aleixo et al., 2015, Li et al., 2013, Liu et al., 2011, Luciano da Silva et al., 2009, Conner, Wolden-Hanson, & Quinn, 2014, Castro & Kuang, 2017, Dougherty, Springer, Gershengorn, 2016, Poole et al., 2020) there may have been inconsistencies with equipment and

schedules perhaps leading to discrepancies. The current study condensed multiple studies exercise protocol's that typically had longer time spans (5 weeks to 12 weeks). Exercise paradigms varied from 16cm/s to 75cm/s all with different durations (2mins to 60mins). There are many exercise paradigms within literature all with different research focuses. Future research could focus on finding an optimal exercise paradigm for stressed mice. Understanding how changing the amount of time animals spend exercising would also greatly benefit future research.

The Harvard treadmill used within this study allowed for mice to see one another side by side on the treadmill. Future research could also investigate if there is a difference between mice being able to see their cage mates and mice that cannot. This would evaluate running environments to see if group exercise would either motivate or distract the mice.

Providing both groups of mice with longer habituation times may also allow researchers to see better effects of exercise. Habituation has been deemed necessary in assisting mice to become comfortable within exercise environments (Svensson et al., 2016). Allowing for a longer habituation time frame to the treadmill apparatus could allow the animals to become more familiar and comfortable with the treadmill and forced exercise. Habituating the animals to the shocks may also help prevent the animals from growing accustomed to the ability to rest at the back on the idle shocks. Another option would be to begin the habituation period with a higher shock voltage. Future research could also focus on the different effects seen within forced and voluntary exercise and how this could affect behaviors specifically within learning and memory.

CONCLUSION

The current study's goal was to investigate the effect of stress and how age and exercise could improve the negative effects of stress. Adolescent mice expressed less anxiety-related behaviors and had higher locomotor activity in comparison to adults within the OFT behavioral test. Adults who exercised expressed more risk taking behavior within the EZM. Adolescents that exercised expressed contradicting results showing less risk taking behaviors.

However, exercised mice showed more defensive behaviors within burrowing behavior. Some of the findings found within this study did not match previous literature that assessed age and exercise, including there being no significant difference between exercise conditions in the MWM. This may have been due to the limitations of the study. Future research should investigate different exercise paradigms on stressed animals and compare the effects of exercise on mice undergoing CMUS administration and their non-stressed counterparts.

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APPENDIX

IACUC Approval



ANGELO STATE UNIVERSITY
College of Graduate Studies & Research
Institutional Animal Care & Use Committee

11-16-2020

Dr. Stephen Lippi
Assistant Professor,
Dept. of Psychology & Sociology
Angelo State University
Member, Texas Tech University System
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San Angelo, TX 76909-0890

Dear Dr. Lippi:

Your proposed project titled, "Assessing the effects of age and exercise on stressed mice" was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective 11-16-2020, and it expires three years from this date; an annual review and progress report form (www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport) for this project is due 11-16 of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 20-203. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Sincerely,

X

Steven T. Brewer, Ph.D.

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